

SKIN TESTING IN 246 PATIENTS WITH NON-SPECIFIC URETHRITIS WITH A REVIEW OF THE IMPORTANT LITERATURE *

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The problem of non-specific urethritis (NSU) is receiving increasing attention, although its existence has been known for over 70 years (Bockhart, 1886), and the recognition of different types of urethritis preceded the discovery of the gonococcus (Neisser, 1879) by an even longer period (Schwedliauer, 1784; Hernandez, 1812; Stevenson, 1823; Parker, 1839).

Waelsch (1901) described a special follicular NSU, which still goes by his name; it is subacute or chronic. Hecht (1927) gave a good account of acute NSU. The only common factor in all these cases appeared to be the absence of the gonococcus.

The clinical problem of NSU came to the fore with the satisfactory diagnosis and rapid cure of gonorrhoea. Up to the present, its infective nature is only apparent on epidemiological investigation, which depends largely on medical history of a particularly unreliable kind as it involves disclosure of venereal exposures. Bound up with this difficulty is the fact that the limits of the incubation period are not known, and a baffling though admittedly small number of patients, who deny firmly any preceding sexual intercourse, can be found in most large series. The few limited epidemiological studies available seem to favour the spread of NSU by sexually active men and women (Durel and Siboulet, 1954). It may be, however, that immunological and microbiological research is more likely to help solve the aetiological problem. Up to date, the main lines of investigations have been concentrated on five possible causes:

- (1) bacteria,
- (2) virus,
- (3) PPLO and "L" forms of bacteria,
- (4) trichomonads,
- (5) primary prostatic disorder.

(1) *Bacterial Aetiology*.—Most bacteria normally found in the lower urogenital tract have been implicated, but it is now generally agreed that only a small proportion

of NSU cases is due to pathogenic strains of bacteria. These could usually be grown in pure culture, and they have responded to specific treatment. The commonest organisms are coagulase positive *Staphylococcus albus* and *aureus*, *Streptococcus faecalis*, haemolytic streptococci, and diphtheroids. Somewhat surprisingly *E. coli* has only rarely been incriminated (Hughes and Carpenter, 1948; Cohn, 1905; Harkness and King, 1938).

More recently, Ambrose and Taylor (1953) reported the presence of small coccobacilli in NSU. Its growth on culture media was strikingly slow, and it may be due to this that the organism was overlooked in routine cultures. Leopold (1953) found microbes related to the genus *Haemophilus*, which he thought to be of aetiological significance.

(2) *Virus*.—Urethritis occurs at times with certain known virus diseases such as measles (Kidd, 1917), herpes simplex (Nicolas, Gaté, and Papacostas, 1923), herpes zoster (Dubois, 1926), dengue fever (Weyrauch and Gass, 1946), mumps (Spence, 1931), lymphogranuloma venereum (Hellerström, 1929), and inclusion blennorrhoea (Harrison and Worms, 1939). Of this heterogeneous group the last named is of the greatest interest, largely due to the work of two ophthalmologists, Lindner and Thygeson, who established a close connexion between inclusion conjunctivitis of infants and adults on the one hand, and certain genital infections on the other.

Lindner (1909, 1911, 1913) demonstrated epithelial inclusions in the genital tracts of mothers whose infants suffered from inclusion conjunctivitis. He then produced experimentally inclusion conjunctivitis in monkeys by inoculating them with vaginal secretions of these women. It was his belief that the infective agent was identical with, or possibly an attenuated, trachoma virus.

The other ophthalmologist, Thygeson (1934), showed that the virus of inclusion conjunctivitis now called *Chlamydozoon oculogenitale*, belongs to the psittacosis-ornithosis-lymphogranuloma group and is distinct from trachoma virus. In animal experiments and epidemiological studies he produced some evidence that inclusion conjunctivitis of infants and also of adults could be caused from minimal genital infections and that the virus was capable of surviving in water for several hours. This led to the idea that the virus may spread from the

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genito-urinary tract to the eyes in non-chlorinated swimming pools, using the water as its vehicle. No evidence has been adduced for infectious spread to the genital tract in swimming pools. Experimentally he was able to produce "inclusion cervicitis" in baboons by inoculations, but failed to provoke inclusion urethritis in either male or female baboons. Thygeson concludes that *C. oculogenitale* causes a benign, chronic, but self-limited urethritis in the male, and an almost symptomless inflammation of the external cervical os in the female. Transmission is by sexual intercourse. Unhappily the virus has not yet been cultivated and apart from the animal transmission referred to, the stained inclusion bodies in epithelial scrapings are the only means of identification (Thygeson; 1934, 1954). Clinically he describes "inclusion urethritis" as a self-limiting minimal urethritis, as a rule without complications, its minimum duration being 5 months, the maximum 11 months (Thygeson, 1954).

It should be mentioned here that we found such a clinical picture amongst our cases, but only in a small number.

(3) *Pleuropneumonia-like Organisms (PPLO)*.—Although these infectious agents were cultivated in 1898 by Nocard and Roux from contagious bovine pleuropneumonia, it was only since Dienes found similar organisms in an otherwise "sterile" Bartholin's abscess (Dienes and Edsall, 1937) that the possibility of its pathogenicity in humans was seriously considered. Dienes and his co-workers were able to show that PPLO were frequent inhabitants of both the male and female genito-urinary tract. At first, as is so often the case, the organisms were reported to occur only in the presence of some infection, notably "Non-gonococcal urethritis" (Beveridge, Campbell, and Lind, 1946; Harkness and Henderson-Begg, 1948). Recently, however, PPLO have been found in apparently normal male urethras and even more frequently in the normal female urogenital tract (Salaman and others, 1946; Harkness, 1950; Randall, Stein, and Ayres, 1950; Melen and Linnros, 1952; Nicol and Edward, 1953). The question of pathogenic and non-pathogenic strains is receiving special attention at present and their position in "non-specific" genital infections is uncertain. Perhaps the most important observation to date is the isolation of pure cultures of PPLO in otherwise "sterile" inflammatory lesions, such as salpingitis, Bartholinitis, and from the synovial fluid of Reiter's arthritis (Dienes, Ropes, Smith, Madoff, and Bauer, 1948; Warthin, 1948). It was apparently the sole organism in some cases of cystitis and pyelitis in men (Dienes and Berg, 1954). An interesting suggestion came from Klieneberger-Nobel (1954) when she described animal experiments with originally non-pathogenic PPLO which became virulent if certain other organisms were also introduced. This activating action deserves further study in man. Willcox (1954b) reported therapeutic success with erythromycin in NSU, and, as PPLO is highly resistant to this antibiotic, its aetiological significance is questioned (Lancet, 1954). Ruiter and Wentholt (1952) instilled PPLO cultures intra-urethrally into two volunteers without any subsequent clinical or bacteriological evidence of infection. They also noted that

abundant growth of PPLO was found only in men with genital infections and, when controls did show the organisms, growth was scanty. In conclusion it may be said that the position of PPLO in human genital infections is unsettled.

When Klieneberger-Nobel isolated a PPLO from a culture of *Streptobacillus moniliformis* in 1935, a different line of investigation was initiated. She called these micro-organisms "L organisms", and it is now generally thought that the L-phase is a variant of the bacterium or part of its life-cycle. Its cultural and other characteristics resemble the true PPLO closely, but it is considered that the risk of confusion is not great (Sorel, 1954; Edward, 1954). A number of bacteria have now been found capable of producing L-forms, but they still retain some of the characteristics of the parent bacterium, e.g., the L-forms of *Proteus vulgaris* possess the characteristic smell (Edward, 1954) and generally the L-phase is difficult to subculture and readily reverts to the bacillary form it stems from. PPLO, on the other hand, are apparently stable. The possibility of gonococci giving rise to L-forms and thus being responsible for NSU was considered by Salaman and others in 1946 when they demonstrated these forms in gonococcal cultures. This suggestion, however, has not found general acceptance, and Salaman himself considered alternative explanations such as double infections.

(4) *Trichomonas Vaginalis*.—This protozoan was first noted in male urethral discharge by Kunstler in 1883 and since that time the percentages of positive findings have varied greatly with different authors (Freed, 1945, 1948). The recent introduction of routine cultural methods in some centres resulted in somewhat higher positive returns; Sorel (1954) observed 11.2 per cent. in 527 patients, but only one of them was acute, the rest with trichomonas having chronic urethritis.

He also found the parasite in the apparently healthy urethras of men whose wives suffered from trichomonas vaginitis. Thus, as with all other alleged causes of non-gonococcal urethritis, the microbial agent has been demonstrated in the apparently healthy urethra.

Lanceley and McEntegart (1953) inoculated cultures of trichomonads intra-urethrally into five male volunteers, all of whom developed urethritis, yet trichomonas could only be recovered in three of them. In conclusion, it is not thought likely on present evidence that trichomonas is a major cause of male urethritis.

Mycotic Infection.—Fungal elements have from time to time been reported in urethral discharges as isolated findings, but recently Auckland and Preston (1954) found them present in 36 out of 722 males with urethral discharge. The possibility of the world-wide use of antibiotics in the last decade, resulting in an increased pathogenicity of fungi, may account for the relatively high proportion found in this series, but there is no proof that the fungi are anything but saprophytic, and in any case they have not been found in important numbers in any other series.

The extent of the problem of NSU in England and Wales may be judged from the Ministry of

Health returns (1954) which have listed NSU as a separate condition since 1951. The number of NSU cases attending the V.D. clinics of England and Wales in 1951 was 10,794 as against 14,975 male gonorrhoea cases; in 1952, 11,552 NSU cases as against 15,510 male gonorrhoea, and in 1953, 13,095 cases of NSU as against 15,258 of male gonorrhoea.

The two sets of figures are in our opinion not strictly comparable for these reasons:

(1) Whereas gonorrhoea is promptly cured by penicillin, leaving a negligible number of relapses, the position is different with NSU; here treatment is less specific and relapses are common. If they happen to follow fresh sexual exposure (and there is some evidence to suggest that even non-infectious intercourse may reactivate latent infection), they are likely to be counted as a new attack of NSU.

(2) The diagnosis of gonorrhoea in the male is straightforward, but that of NSU depends on negative properties and is thus less easily definable.

In addition, NSU has a wider range of severity than gonorrhoea, with a special tendency to minimal and intermittent signs. Some of these may in fact not be NSU, but may be due to increased secretions of an otherwise normal genital tract. This is commonly associated with venereophobia. The distinction, however, is not always clear and diagnosis will vary with the inclination of the physician concerned. Interesting numerical expression of the problem can be found in the classification of a large series of "urethritis" (Durel and Siboulet, 1954) of 2,000 patients attending male urethritis clinics:

Disease	No. of Cases (per cent.)
Gonorrhoea	40
NSU	23.6
Unimportant discharges and gonophobia	30.6

Thus the "unimportant" discharges constitute a sizable group, and it is plausible that, until we have a certain way of diagnosing NSU, its incidence cannot be accurately determined.

With the strongly held rival views on the causation of NSU it is not surprising that at the Symposium on Non-Gonococcal Urethritis held at Monaco (1954) the question of aetiology was kept open without any line of investigation having been found more promising than any other. In fact, some inclined to the view that the causes may be many, even though clinical differentiation is as yet not possible.

The recent series of patients who have been investigated in order to find the major aetiological

factor, mainly by workers in the United States, France, and Great Britain are set out in Table I (opposite).

The present position with respect to NSU may be summarized as follows:

(i) Infection is the probable cause but has not been proved for the majority of cases;

(ii) Epidemiological studies have not been used on a scale large enough to extract all the information they are capable of giving;

(iii) Experimental NSU has not yet been produced in men with any of the suggested infective agents except for the small series with *Trichomonas vaginalis*;

(iv) *C. oculogenitale* has defied culture so far;

(v) The position of PPLO and "L" forms is uncertain in the aetiology of any human infection; the question of pathogenic strains and of activating factors being involved in changing harmless strains into virulent ones are problems receiving increasing attention; and the fact that PPLO are present in many of these cases in abundant culture has been stressed (Ruiter and Wentholt, 1953);

(vi) The thesis of a pre-existing quiescent inflammation of the peri-urethral glands leading to a descending urethritis has been again put forward as a major cause of NSU and needs careful consideration;

(vii) The suggestion by Coutts (1937, 1948) and Atlas (1948) that a spirochae'te may play a responsible role in the aetiology has not been favoured;

(viii) Reiter's syndrome has been considered by many to be very closely related to NSU; the subject has been fully reviewed by Harkness (1950) and Daguet (1952).

SKIN TESTS

Skin tests have been used in this study to determine whether an altered skin sensitivity existed in NSU, and, if so, to discover whether this skin response was of a specific nature.

The mechanisms of immunity and hypersensitivity, whereby the body responds to the challenge of infecting agents, have been studied and made use of in medicine for a long time.

In the field of venereal infection one is reminded of the pioneering work with skin tests of Frei (1925) in lymphogranuloma venereum, Noguchi (1911) and Kolmer and Greenbaum (1922) in syphilis, Engel and Grundmann (1933) in gonorrhoea, Ito (1913) and Reenstierna (1924) in chancroid, Kornblith (1944) in granuloma inguinale, and Adler and Sadowsky (1947) in *Trichomonas vaginalis*.

The many factors which affect the results in skin testing in infectious disease have been summarized by Beerman and Ingraham (1950). On the technical side certain factors make standardization of the skin test very difficult, e.g., the non-specific response of the skin to animal tissue, as with mouse brain tissue in the Frei test; the concentration of antigen; dosage; times of reading the tests; definition of test results.

TABLE I
RECENT MAJOR WORK ON THE AETIOLOGY OF NSU

Aetiological Agent under Investigation	Authors	Year	No. of Patients	Results
1. Bacteria *	Hughes and Carpenter	1948	117	All thought to be "bacterial"
	Harkness	1950	144	25.5 per cent. thought to be "bacterial"
	Willcox	1954a	81 NSU 105 Controls	No significant difference bacteriologically between the two groups
2. <i>Trichomonas Vaginalis</i>	Freed	1948	112	28.5 per cent. positive
	Sorel	1954	527	11.2 per cent. positive
	Lanceley and McEntegart	1953	310	5.3 per cent. positive
	Durel and Siboulet	1954	412	10.2 per cent. positive
3. Pleuropneumonia-like Organisms (PPLO)	Harkness and Henderson-Begg	1948	839 NSU 139 Gonorrhoea 50 Male controls	16 per cent. positive 9 per cent. positive 0 per cent. positive
	Melen and Oberlad	1952	61 NSU 60 Controls	18.1 per cent. positive 16.6 per cent. positive
	Durel and Siboulet	1954	631 NSU	7.4 per cent. positive (same proportion of PPLO in urethritis of all degrees)
	Nicol and Edward	1953	140 NSU 110 Male controls 35 Cervicitis 40 Female controls	25.7 per cent. positive 12.7 per cent. positive 48.5 per cent. positive 22.5 per cent. positive
	Shepard	1954	42 NSU	71 per cent. positive
	Brisou	1954	350 NSU	12.7 per cent. positive
	Dienes and Berg	1954	86 NSU 67 Controls	64 per cent. positive 27 per cent. positive
	Randall and others	1950	300 Cervical cultures	26 per cent. positive
4. Inclusion Urethritis	Bedson	1950	25 NSU	None positive
	Thygeson and Stone	1942	100 NSU and Gonorrhoea	8 per cent. positive
	Durel and Siboulet	1954	2,328 NSU	3.5 per cent. positive
	Willcox	1954a	250 NSU 108 Gonorrhoea	27.6 per cent. positive 21.7 per cent. positive
5. Fungi	Auckland and Preston	1954	602 NSU 120 Gonorrhoea	5 per cent. positive 5 per cent. positive
6. Primary Prostatic Diseases	Ambrose and Taylor	1953	Considered that 25 to 30 per cent. of young males have primary, silent chronic prostatic infection causing secondary urethritis	
	Graham	1954	Believed that a prostatic dysfunction is present in a large proportion of patients who show recurrent infection of the urethra	

* The coccobacillus (Ambrose and Taylor, 1953,) and haemophilus (Leopold, 1953) isolated in cases of NSU are too recent and unconfirmed to be included.

According to Beerman and Ingraham, three types of skin test reaction may occur: a rapid histamine-like reaction, delayed or tuberculin-like reaction, or a late eczematous reaction. The second type, due to tissue allergy, is the commonest.

Skin tests have been used before in the study of NSU and Reiter's syndrome, but their use has been limited to a few cases. Harrison and Worms (1939), in their review of the problem of inclusion urethritis, remind us that Frei, Wiese, and Klestadt (1932), Kalz (1933), and Bezecny (1934) all showed that an allergic reaction could be obtained in lymphogranuloma patients using urethral discharge from a case of Waelsch urethritis.

Conversely, allergic skin reactions in a patient with Waelsch urethritis could be obtained using Frei antigen (Bezecny 1934; Fahlbusch and Zierl, 1937; Bizzozero and Midana, 1938; and Ross, 1939).

More recently Storm-Mathisen (1946), Thiers and Joly (1948), Harkness and Henderson-Begg (1948), and Thygeson (1954) have reported similar work. Storm-Mathisen used gland emulsion and joint exudate as the antigen for skin testing, Thiers and Joly used urethral pus, Thygeson urethral scrapings, and Harkness and Henderson-Begg phenolized suspensions of "L" organisms. Very few cases were investigated in this way, and only Thygeson reported completely negative results.

Method

In this study the antigen for skin testing was prepared from the urethral secretions of patients with non-bacterial urethritis. The platinum loop scraping was mixed with normal saline. This mixture was first subjected to high speed centrifugation for 15 min., and the supernatant fluid was then withdrawn and incubated at 60° C. for 1 hr. Before use the fluid was tested for bacteriological sterility, and 0.5 per cent. phenol was added.

For the skin test, 0.2 ml. was injected intradermally in the forearm, and the result was read after 48 hrs. The test was considered positive when there was a papule with erythema of 1 cm. or more in diameter. Each fresh batch of skin test material was, of course, of unknown antigenic potency and had to be tested against an already proved sensitive patient for its capabilities to be known. It was this problem which made the process difficult and used valuable quantities of meagre skin test material. For this reason, many of the lots were pooled before use.

The donor material was made up at two centres and exchanged by the authors, until it was clear that similar results were being obtained with each group of materials.

Control over the donor skin test material was maintained by :

- (i) using a control injection of sterile 0.5 per cent. phenol in normal saline ;
- (ii) preparing antigen in a similar fashion from cases of acute gonorrhoea ;
- (iii) skin testing a group of patients who did not have NSU.

This control group was largely made up of cases of acute gonorrhoea, since it was considered very desirable to try to eliminate the possibility of a non-specific response to urethral tissue.

Results and Discussion

In the assessment of this experiment 246 cases were used. The ages of the three groups of patients, NSU, gonorrhoea, and non-venereal, demonstrated the slight bias, noted by others, in NSU towards the less young patient as compared with acute gonorrhoea.

In the case of the married patient, positive skin reactions occurred with equal frequency in those who had, as those who had not, a history of extra-marital exposure. The disease did not favour either the single or the married man ; there were 105 single and 58 married with NSU in the study.

It has often been said that these patients include a high proportion of men who indulge in excessive

numbers of risks, or in excessive intercourse with the female. This was not the finding of this study.

The incubation periods disclosed a wide variation, but many of the apparently shorter incubation periods may well have been longer owing to failure of the patient to declare every risk.

It is noteworthy that many authorities consider that the longer incubation period belongs to the commoner type of NSU. The accompanying figures demonstrate the inconclusiveness of this factor.

Incubation period (weeks)	1	2	3	4
No. of patients	21	19	21	34
Skin tests positive (per cent.)	67	70	70	73

A positive skin reaction was obtained quite early in the patient's disorder, the majority being tested within the first few days of attendance.

Positive reactions unexpectedly tended to occur more frequently in those patients who responded more rapidly to treatment. The skin test results were tabulated against the response to treatment in 162 patients, 107 of whom responded well and 55 badly. The standard therapy for purposes of the study, which is not considered more than 55 per cent. efficient by Harkness, was streptomycin in conjunction with sulphonamides. Positive reactions were noted in 46 per cent. of the patients who responded well, and in 36.4 per cent. of those who responded badly to therapy, and were negative in 21 per cent. and in 33 per cent. respectively. These figures, although not really significant, are suggestive.

Similarly, 94 results were arranged so that the response to treatment was set against incubation periods (considered as either of the shorter or longer type) ; 23 cases had an incubation period of 10 days or less, and 71 of over 10 days. Seventy-nine per cent. of those with the shorter incubation period responded to treatment rapidly, 60 per cent. giving a positive, and 30 per cent. a negative skin reaction. Of those with the longer incubation period, approximately equal numbers responded rapidly and failed to respond to therapy, 55 per cent. giving fully positive and 15 per cent. negative results.

Of seven cases whose donor skin test material was used unpooled and alone, in whom a complete record of their results was available, the incubation periods lay between 14 and 26 days ; they all responded positively to the skin test, and their treatment responses (rapid or poor) were equally divided.

Fairly extensive multiple testing of the individual patient was undertaken in order to test the patient's ability to retain the allergy. Some cases, who

started negative, did not show a positive response until the second or third week. The skin response remained positive in some cases for many weeks and seemed to become negative within 2 months. There were many exceptions, and the numbers were too small to draw hard conclusions. The cases who were negative at the end of the second week more often than not remained so.

Table II shows the results of the experiment as a whole. In 137 cases of NSU, 53.3 per cent. gave fully positive results, and 30 per cent. negative. If the weakly positive results are added in turn to the positive and negative groups it appears that at best 70 per cent. and at worst 47.5 per cent. gave positive skin test reactions. The insertion of the doubtful NSU group gives some idea of the usefulness of this type of test in an incompletely diagnosed case.

TABLE II
DETAILS OF THE SKIN TEST RESULTS IN 246 CASES

Disorders	Number of Patients		
	Positive	Weak Positive	Negative
NSU	72	24	41
Probable NSU .. .	11	3	11
Gonorrhoea .. .	1	3	30
Gonorrhoea + NSU (double infection) .. .	3	1	2
Reiter's syndrome .. .	3	1	4
Others * .. .	1	0	35

* Healthy patients, venereophobia, syphilis, lymphogranuloma venereum, and general medical cases.

Weight was added to the overall figure for skin reactions in NSU when it was apparent that the results from the three centres concerned in the work were very similar (Table III).

TABLE III
DETAILS OF SKIN TEST RESULTS IN NSU FROM THREE INDIVIDUAL CENTRES OF STUDY

Hospital	Number of Patients		
	Positive	Weak Positive	Negative
Seamen's .. .	20	2	12
St. Mary's .. .	25	10	16
Guy's .. .	27	12	13

The skin test was positive in several cases of acute gonorrhoea that did not respond to the specific therapy for that disease. In typical cases of gonorrhoea there were negligible reactions.

The fact that about half the small number of cases of Reiter's syndrome responded to the skin

test was not unexpected. Many workers have demonstrated a skin test response in these cases. The fact that the arthritic syndrome rarely follows immediately upon the urethritis may have some significance.

The heterogeneous group of control cases in Table II contained only one reactor.

Antigen for skin testing, made up from cases of acute gonorrhoea, and tested in over two dozen patients, resulted in one positive and one weak positive reaction in gonorrhoea, and one positive and one weak positive in NSU.

The local application of steroid hormone (hydrocortisone ointment, 1 and 2½ per cent.) was undertaken in 39 cases of all types of NSU. There was a striking symptomatic improvement in almost every case, and the meatal epithelium appeared less red and moist. The urine however contained a first glass haze and debris, and a return of the urethral discharge was either immediate or delayed a week or so following cessation of therapy. In a few cases inclusions were noted in the scrapings from the urethra after steroid therapy when they had not been noted before. This requires further study.

What was the nature of the allergen in the skin test? Since the controls gave practically no response, it is thought unlikely that a non-specific reaction underlies these results. Two known allergens, lymphogranuloma venereum, and *Trichomonas vaginalis* in the female, produce positive skin test responses. On present evidence, virus infection or an infection in which pleuropneumonia-like organisms have a role would seem the most likely explanation of the high proportion of skin test reactions.

SUMMARY

The results of a skin testing experiment in 246 patients is described. In 137 cases of NSU and seventy controls between 50 and 70 per cent. of cases of NSU gave positive reactions, whereas a very small number of controls were positive to the test.

It is suggested that between 50 and 70 per cent. of cases of NSU are due to a predominating cause, and that an infecting agent in all probability exists in these cases.

The skin test reactions were of the delayed or tuberculin type. A few cases of the immediate type of reaction were noted but not included.

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